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Honey bee venom promotes the immune system and reduces *Vairimorpha (Nosema) ceranae* infection in honey bees (*Apis mellifera* L.)

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Abstract – Bee venom (BV) extraction has become a standardized practice, characterized by being safe, simple, and causing no harm to bees. Though it demonstrated significant antimicrobial activity, its potential activity against honey bee pathogens have yet to be investigated. Therefore, we investigated for the first time in a controlled laboratory setting the effectiveness of BV administration in sugar syrup against *Vairimorpha (Nosema) ceranae*, a globally widespread fungal disease of honey bees. To do this, we first determined the BV lethal concentration (LC₅₀), after which newly emerged bees were inoculated with *V. ceranae* spores (10⁵) or/and chronically fed sugar syrup containing BV at LC₁₀ (0.24 mg/mL) or LC₂₀ (0.34 mg/mL) ad libitum for 12 days. The effects on bee longevity (time to death) were studied. The intensity of *V. ceranae* and total hemocyte count (THC) were also studied 6 and 12 days after infection. The expression of four antimicrobial peptides (AMPs) encoding genes (defensin1, apismin, hymenoptecin, and PGRPS2) was also measured after 6 days in midgut tissues. We discovered that BV increased bee survival and significantly reduced *Vairimorpha* intensity. This effect was associated with an increase in THC as well as the expression of the AMP-encoding genes studied. BV's effects appeared to be concentration- and time-dependent, with a more profound decrease in *Vairimorpha* intensity at day 12, especially in bees fed BV at LC₂₀. These findings highlight BV's potent immunomodulatory role against fungal pathogens in honey bees, and its potential role against other bacterial and viral pathogens should be investigated.

bee venom / honey bees / antimicrobial peptides / cellular immunity / antifungal activity / nosemosis

1. INTRODUCTION

The Western honey bee (*Apis mellifera* L.), which ranks as the most frequent single crop pollinators worldwide, offers highly valued services for the agriculture sector (Al Naggar et al. 2018; Garibaldi et al. 2013; Khalifa et al. 2021). It also produces high-quality food, such as honey, royal

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jelly, and pollen, as well as beeswax, propolis, and bee venom (BV). However, the decline in bee populations, particularly in the USA and Europe, has raised concern among scientists, governments, and the general public since 2006 (Lima et al. 2022; Phiri et al. 2022). A variety of biotic and abiotic factors have been identified as potential contributors to the colonies loss (Al Naggar et al. 2022; Hristov et al. 2020; Insolia et al. 2022; Jaffé et al. 2019; Lima et al. 2022), whereas parasites and pathogens pose the greatest threat (Al Naggar and Paxton 2020; Brosi et al. 2017), and finding control strategies have long been a request from beekeepers (Dietemann et al. 2012).

Given that host population density shapes parasite and pathogen spread as well as epidemic size, it is a major contributor to increased parasite and pathogen prevalence and incidence in honey bees, in addition to the high temperature and humidity levels maintained in a bee nest (Baracchi et al. 2012; Brosi et al. 2017). As a result, honey bees have developed a number of strategies and adaptations to reduce this risk (Wilson-Rich et al. 2009). Propolis and gland secretions are examples of natural antimicrobial substances that honey bee species collect and use to reduce pathogens risks (Simone-Finstrom and Spivak 2010; Stow et al. 2007; Zasloff 2002). A significant source of antimicrobial substances has recently been found in the venom gland of honey bees and other Hymenoptera (Kuhn-Nentwig 2003). As a result, some researchers hypothesized that BV aids in the development of social immunity in bees because antimicrobial venom peptides have been discovered on the cuticle of adult bees and on comb wax, since the wax comb can act as a social antiseptic device by substituting for the “medium” where the venom is deposited (Baracchi et al. 2011).

BV is a complex mixture of proteins (phospholipase A and hyaluronidase), peptides (melittin and apamin), physiologically active amines (histamine and dopamine), sugars, phospholipids, and volatile compounds (Wehbe et al. 2019). The most active compound, with up to 50% dry weight, is melittin. Its high antibiotic and biological activity have been studied for many decades in human apitherapy (Askari et al. 2021; Duffy et al. 2020; Rady et al. 2017; Weis et al. 2022).

Though BV’s antimicrobial activity against bacteria, viruses, and fungi has been demonstrated in vitro and in vivo experiments (reviewed in El-Seedi et al. 2020), little is known about BV’s ability to combat honey bee diseases.

Previous research revealed that BV had in vitro activity against *Paenibacillus* larvae, the causative agent of American foulbrood (AFB), one of the most destructive *A. mellifera* brood diseases (Fernández et al. 2014). Recently, in *Varroa* mite-infected colonies, BV and Egyptian propolis extract (EP) were used as supplements in the nutrition at different concentrations to measure the colonies’ activities after treatment. Treated colonies with BV and EP increased the expression of immune-related genes (defensin, abaecin, hymenoptaecin, and vitellogenin) and increased the life span, activities, and density of bee workers (Seyam et al. 2022), implying that BV plays an important role in promoting bee immunity and health. As a result, it is critical to investigate the potential role of BV in protecting honey bees from viral and fungal diseases.

The immune system in insects is made up of both cellular and humoral components. Hemocytes are involved in the cellular component (Winson 1993), and total hemocyte count (THC) is an indirect measure of basal cellular immunocompetence because it is involved in processes such as phagocytosis and parasite encapsulation, both of which have been observed in insects against fungal pathogens infestation (Hillyer 2016). In the current study, we investigated for the first time the effectiveness of BV against *Vairiormorpha* (*Nosema*) *ceranae*, a globally widespread fungal disease that infects and multiplies in the midgut epithelia of adult honeybees. The severe infestation has been linked to shorter forager lifespans and the increase of colony mortality (El Khoury et al. 2018; Gage et al. 2018; Higes et al. 2007). To do this, newly emerged bees were infected with *V. ceranae* spores and fed sugar syrup containing BV at LC₁₀ or LC₂₀ levels either individually or in combination. The effects on survival, *V. ceranae* intensity, total hemocyte count (THC), and expression of some AMP-encoding genes were studied. We hypothesized that BV would promote bee immunity and health, thereby reducing infection intensity.

2. MATERIALS AND METHODS

2.1. Sources of honey bees and bee venom

Experiments were conducted during May–August 2022 with *A. mellifera* workers of the subspecies *Carnica* obtained from hives of the apiary of the experimental farm, city of scientific research and technological applications (SRTA-city) (New Borg El Arab, Alex., Egypt). Colonies had no visible honey bee diseases, sufficient bee bread and honey stores, and a large number of nurses and honey bee workers, with each colony consisting of eight bee-covered combs, including two to three brood combs. The honey bee venom (BV) used in this study (1 g) was obtained from a private beekeeper in Egypt's Al Gharbia governorate through an electro-stimulated extraction as described in the standard methods for *Apis mellifera* venom research (de Graaf et al. 2021) and dissolved in 100 ml of distilled water and stored at $-20\text{ }^{\circ}\text{C}$ for short-term (up to 6 months) or lyophilized at $-80\text{ }^{\circ}\text{C}$ for extended storage for more than 1 year (de Graaf et al. 2021).

2.2. Determination of LC_{50} of BV and the used concentrations

Sealed brood frames from three different colonies have been selected and transferred to carton boxes and kept at room temperature ($31\text{--}33\text{ }^{\circ}\text{C}$, $60 \pm 10\%$ Rh.). The next day, newly emerging bees < 24-h old were collected for use in the experiments. Six serially diluted concentrations ($10\times$) of BV were prepared in 50% (w/v) sugar solution (0.0001–10 mg/ml) were tested against honey bee workers to determine the limit range of toxicity. Then, another range of five serially diluted concentrations were used (0.1, 0.3, 0.6, 0.9, and 1.2 mg/ml) to determine the median lethal concentration (LC_{50}) of BV. Dimethoate insecticide was used as a positive control insecticide at the recommended concentration $1.5\text{ }\mu\text{g/ml}$ to verify the exposure conditions. Adult workers were transferred to ventilated wooden cages ($9.5 \times 4 \times 7.5$

cm), with 15 bees per cage and three cages per concentration. Bees were deprived of food for 2 h prior to exposures, and thereafter sucrose solution spiked with BV at the desired concentration or control sucrose solution. Access to sucrose solution was through a 15-mL falcon tube placed at the top of each cage. Mortality was recorded after 72 h, and LC_{50} , LC_{20} , and LC_{10} for BV was then calculated using the LdP LineR program using the log-probit model (Ehabsoft, <http://www.ehabsoft.com/ldpline>).

2.3. *Vairimorpha* (*Nosema*) spores preparation

Vairimorpha spores were obtained from highly infected colonies, as previously described (Fries et al. 2013). *Vairimorpha ceranae* spores used in bioassays were first propagated in the laboratory through mass feeding of caged honey bees with *V. ceranae* spores originating from infected bees collected from the apiary of the experimental farm, city of scientific research and technological applications (SRTA-city) (New Borg El Arab, Alex., Egypt). Inocula were prepared freshly on the day of experimental bee infection by crushing the ventriculus of infected honey bees in distilled water and purifying using Percoll® following standard procedures (Fries et al. 2013). Spore numbers were counted with a Neubauer improved hemocytometer (LO-Laboroptik Ltd, Lancing, UK) under a light microscope ($\times 400$) and diluted to obtain the required concentration (10^4 per μL) in 50% (w/v) sucrose solution. For the control treatment, an extract from the ventriculus of uninfected caged honey bees was obtained as above.

2.4. Honey bee exposure to BV and *Vairimorpha* (*Nosema*) *ceranae*

BV1 (LC_{10} : 0.24 mg/mL) or BV2 (LC_{20} : 0.34 mg/mL) was tested against *V. ceranae*. Frames of honey bee brood ready to emerge were collected from three *V. ceranae*-free colonies and kept

overnight in carton boxes at room temperature (31–33 °C, 60 ± 10% Rh.). Freshly emerged honey bee workers were exposed to BV1 or BV2 either individually or combined with infection with 10⁵ spore of *V. ceranae*. The number of spores used to infect bees (10⁵ per bee) was chosen to ensure infection of every individual (Forsgren and Fries 2010; Mura et al. 2020). To do this, we starved bees for 2 h and then hand fed bees with 10-µl inoculum containing either 10⁵ *Vairimorpha* spores (*V. ceranae*, BV1 + *V. ceranae*, and BV2 + *V. ceranae*) or gut homogenate free of *Vairimorpha* spores (Control, BV1, and BV2). As a result, bees were randomly assigned to one of six treatment groups: control, BV1, BV2, *V. ceranae*, BV1 + *V. ceranae*, and BV2 + *V. ceranae*, with 30 bees per cage and three cages per treatment. Cages were kept at room temperature (31–33 °C, 60 ± 10% Rh.) and provided with sugar water ad libitum (control and *Vairimorpha*), or sugar water containing BV1 (BV1 and BV1 + *V. ceranae*) or BV2 (BV2 and BV2 + *V. ceranae*) for 12 days. After the spores were purified, it took approximately 4 h to prepare the treatments and administer them to the bees. Dead bees were recorded and removed daily till the death of all bees in experimental groups. At days 6 and 12 post treatment, four surviving bees from each cage (12 bees per treatment) were collected to quantify *Vairimorpha* infection intensity and to investigate effects on total hemocyte count and expression of immune-related genes, as described below.

2.5. Quantification of *Vairimorpha* (*Nosema*) intensity in infected bees

We counted the number of *Vairimorpha* spores in the midguts of bees using six randomly selected workers per treatment at 6 and 12 days after treatment. To do this, we dissected their midguts into a single-Eppendorf tube containing 1 mL of deionized water as previously described (Fries et al. 2013). Then, using a Neubauer-improved hemocytometer (LO-Laboroptik Ltd, Lancing, UK) and a light microscope (400), we quantified the number of spores in a 10-µL subsample. The total number of spores per sample was then calculated.

2.6. Total hemocyte count

Total hemocyte counts (THC) were performed using the same bees used for *Vairimorpha* spore counts (THC). THC was performed as described by Jones (1962) and Tauber and Yeager (1935). Total hemocytes were counted with improved Neubauer hemocytometer. One microliter of hemolymph per bee was drawn from the bee thorax into Thoma white blood cell diluting pipette up to 0.5 mark and then diluted 20 times with Toissin's solution (1 g NaCl, 8 g Na₂SO₄, 30 mL neutral glycerine, 0.025 g methyl violet, and 160-mL distilled water) up to mark II. After discarding a few drops, one drop was placed near the edge of cover slip of Neubauer ruling and counting chamber automatically filled by capillary action. Four white-cell squares from both upper and lower chambers were counted. Total hemocytes per cubic millimeter were calculated by using formula suggested by Jones (1962).

2.7. Gene expression of immune-related genes

We used real-time quantitative PCR (RT-qPCR) to measure for any effects of BV and/or *V. ceranae* exposure on the expression of four key immune genes that have been used for comparable studies in the past (Aufauvre et al. 2014; Hu et al. 2017). These genes, which are all part of the toll/antimicrobial peptide or the Imd pathway and have been implicated in insect immune responses, include defensin1, apismin, hymenoptecin, and PGRPS2 (Al Naggat and Baer 2019; Evans et al. 2006; Yang and Cox-Foster 2005). The sequences of these primers are shown in (Table I).

Total RNA was isolated from alimentary canals (midgut) of worker bees by use of an RNA extraction kit (Thermo scientific) according to the manufacturer's protocol. Concentrations of RNA were determined by use of a Nanodrop ND-1000 spectrometer (Nanodrop Technologies, Wilmington, DE). The RNA samples were quantified in a single-step assay after the normalization of certain concentrations using 2×SYBER Green qRT-PCR Master Mix kit (Applied Biotechnology, Egypt). β-actin was used as a reference or housekeeping gene for normalizing

Table I Primer sequences used for the quantification of abundances of transcripts in honeybees (*Apis mellifera*)

Gene name	Sequence 5'–3'	Reference
Peptidoglycan-recognition protein (PGRPS2)	F: 5'-TAATTCATCATTCCGGCGACA-3' R: 5'-TGTTTGTCCCATCCTCTTCC-3'	(Hu et al. 2017)
Defensin-1	F: 5'-TGCCTGCTACTGTCTCAG-3' R: 5'-AATGGCACTTAACCGAAACG-3'	(Hu et al. 2017)
Hymenoptecin	F: 5'-CTCTTCTGTGCCGTTGCATA-3' R: 5'-CGTCTCCTGTCATTCCATT-3'	(Hu et al. 2017)
Apismin	F: 5'-TGAGCAAAATCGTTGCTGTC-3' R: 5'-AACGACATCCACGTTTCGATT-3'	(Hu et al. 2017)
β-actin (host reference)	F: 5'-ATGCCAACACTGTCCTTTCTGG-3' R: 5'-GACCCACCAATCCATACGGA-3'	(Aufauvre et al. 2014)

RNA levels of the target genes. Amplification reactions were performed as described in details (Elkobrosy et al. 2022). In brief, a Thermo Rotor-Gene Q 560-system (Qiagen, Germany), and 1.5 μ L (0.1 mM) of each primer (forward and reverse) (Metabion International AG, Germany), 1 μ L (10 ng) template RNA, 8.5- μ L distilled sterile water, and 12.5- μ L qPCR SYBER Green Master Mix (2 \times) (Applied Biotechnology, Egypt) were added to make a final volume of 25 μ L. Amplification reactions were performed in a 36-well Thermo Rotor-Gene Q qRT-PCR system. The manufacturer's recommended universal thermal protocol used includes 3-min activation of thermo-start at 95 °C followed by 15 s at 95 °C for initial template denaturation, 45 cycles of 60 °C for 30 s each, 30 s at 55 °C of annealing and extension phase of 72 °C for 30 s. Three composite samples ($n=3$) from each treatment were used in total for the qPCR experiment, which was only done on day 6. Fold changes of genes of interest were analyzed by use of the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

2.8. Statistical analysis

All statistical analyses and data visualizations were performed in GraphPad Prism 8.0 (GraphPad Software, La Jolla California USA, www.graphpad.com). Normality of data was assessed by use of the Kolomogrov-Smirnov test, and homogeneity of variance was assessed by use of

Levene's test. To meet normality assumptions, gene expression data were log-transformed. Right-censored samples (bees removed on day 6) were recorded in the dataset and incorporated in the Kaplan–Meier log-rank paired tests to compare survival between treatments. Differences in *Vairimorpha* spore count among treatments were done using Student's *t*-tests. Differences in total hemocyte count and gene expression abundances of genes involved in immunity were assessed using one-way ANOVA followed by Tukey's post hoc test, $P < 0.05$, respectively. For all tests, an alpha level of 0.05 was used.

3. RESULTS

3.1. Determination of BV LC₅₀

The acute oral toxicity of BV to newly emerged workers after 72 h of exposure revealed a BV LC₁₀ of 0.24 mg/mL, LC₂₀ of 0.34 mg/mL, and LC₅₀ of 0.66 mg/mL with lower and upper limits of 0.16–0.31, 0.25–0.41, and 0.56–0.77, respectively (Figure S1).

3.2. Effects on survival

We found a significant reduction in survival of bees infected with *Vairimorpha* spores

compared to control and bees fed BV at LC₁₀ or LC₂₀ (log-rank (Mantel-Cox) Test, $p < 0.05$; after Bonferroni correction (Figure 1)), while we found a significant increase in survival of bees infected with *Vairimorpha* and fed BV at LC₁₀ or LC₂₀ compared to *Vairimorpha*-infected bees (log-rank (Mantel-Cox) test, $p < 0.05$; after Bonferroni correction (Figure 1)), implying that BV had the ability to reduce *Vairimorpha* virulence in honey bees. There was no significant difference in survival of bees chronically fed sugar syrup containing BV1 or BV2 for 12 days compared to controls ($p > 0.05$).

3.3. Effects on *Vairimorpha* (*Nosema*) infection

When we compared the infection intensity in bees infected with *Vairimorpha* and fed sugar syrup

containing BV1 or BV2 at 6-, and 12-day post-infection to bees infected with *Vairimorpha* only, we found a significant reduction (Student's *t*-test, $p < 0.01$) in *Vairimorpha* intensity in both timelines, with more profound decrease at day 12, especially in bees fed BV2 (LC₂₀) (Figure 2), demonstrating BV's potent antifungal activity against *Vairimorpha*. As expected, non-infected groups (control and BV-fed bees) were free of *Vairimorpha* infections.

3.4. Effects on total hemocytes count

We found a significant increase in THC in bees fed sugar syrup spiked with BV1 (LC₁₀) only after 12 days of exposure compared to control bees, while bees fed BV2 at LC₂₀ had a significant increase ($p < 0.05$) in THC at 6 and 12 days of exposure compared to control (Figure 3). This trend was reversed in

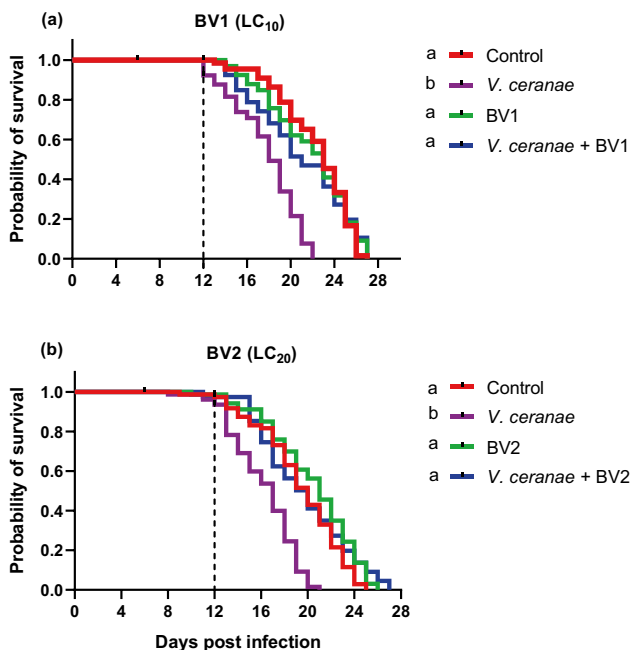


Figure 1. The Kaplan–Meier survival curves in days post-infection of the impact of the gut parasite *Nosema ceranae* and honey bee venom (BV), alone and in combination on adult honey bees. Bees ($n=30$ bees per cage, $n=3$ cages per treatment) were fed 10 μ l sugar syrup containing 10^5 *Vairimorpha* (*Nosema*) *ceranae* spores and then fed sugar syrup containing BV1 (LC₁₀: 0.24 mg/mL) (a) or BV2 (LC₂₀: 0.34 mg/mL) (b), or a control solution. *Vairimorpha* spores were inoculated once at day 0, while BV was fed ad libitum for only 12 days (vertical dashed line). Different lowercase letters indicate statistical differences between treatments after Bonferroni correction (log-rank (Mantel cox) paired test, $p < 0.008$).

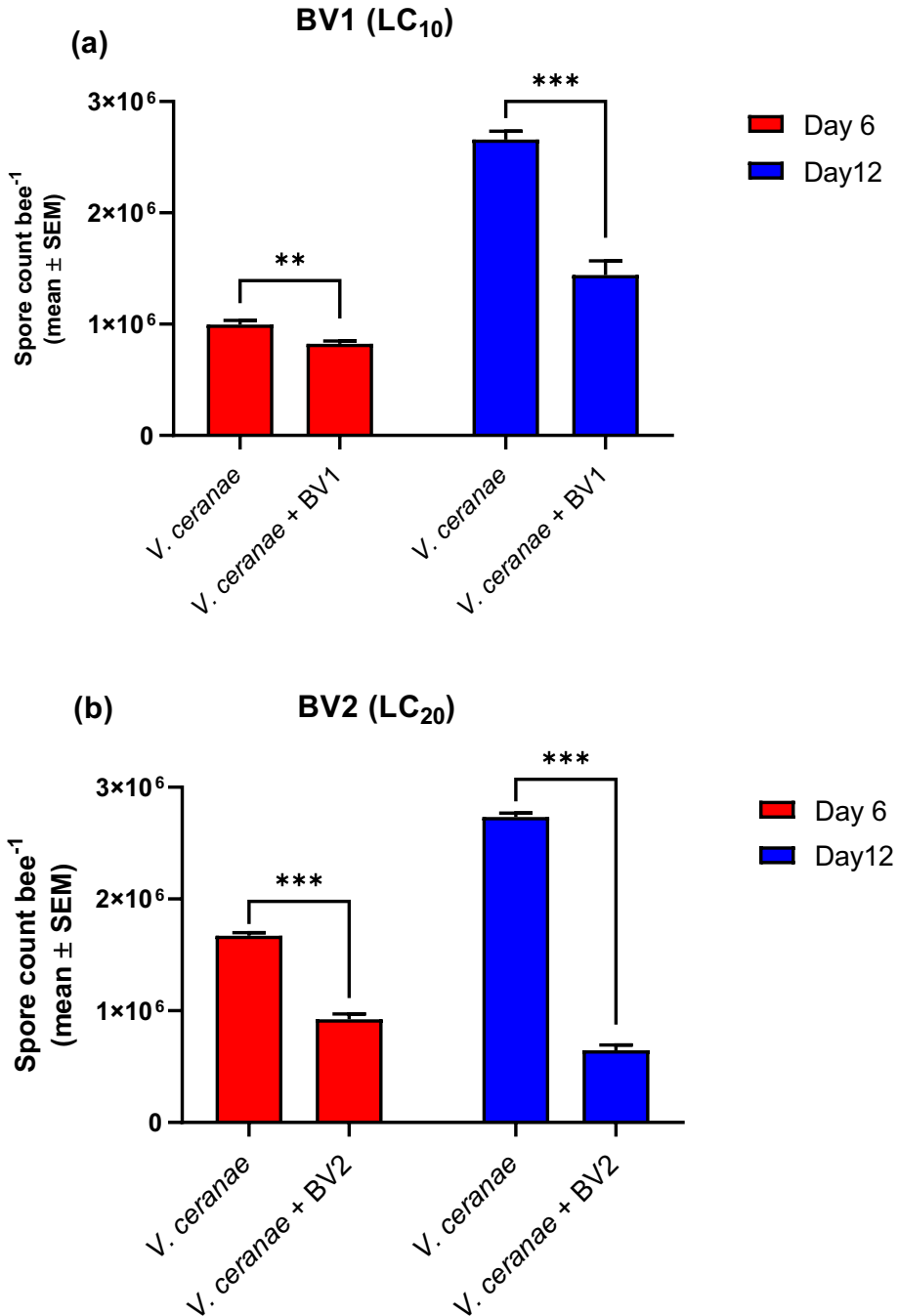


Figure 2. *Vairiormorpha (Nosema) ceranae* infection intensities (mean ± SEM, $n=6$) between treatments at 6- and 12-day post-infection. Bees ($n=30$ bees per cage, $n=3$ cages per treatment) were fed 10 μ l sugar syrup containing 10^5 *Vairiormorpha (Nosema) ceranae* spores and then fed sugar syrup containing BV1 (LC₁₀: 0.24 mg/mL) (a) or BV2 (LC₂₀: 0.34 mg/mL) (b), or a control solution. *Vairiormorpha* spores were inoculated once at day 0, while BV was fed ad libitum for 12 days. The asterisk indicates a significant decrease in spore count treatments at 6- and 12-day post infection (Student's *t*-test, ** $p < 0.01$, and *** $p < 0.001$).

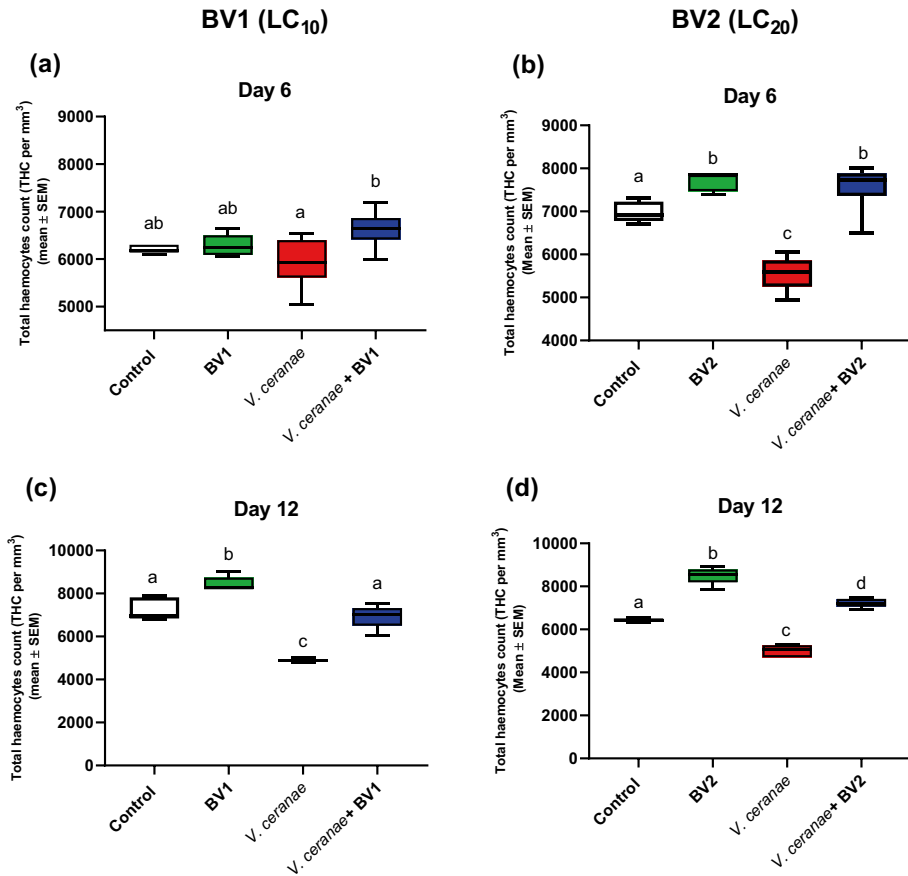


Figure 3. Total haemocytes count (THC) ($n=6$) in different treatments at 6- and 12-day post-infection. Bees ($n=30$ bees per cage, $n=3$ cages per treatment) were fed 10- μ l sugar syrup containing 10^5 *Vairiomorpha* (*Nosema*) *ceranae* and then fed sugar syrup containing BV1 (LC₁₀: 0.24 mg/mL) (a, c) or BV2 (LC₂₀: 0.34 mg/mL) (b, d), or a control solution. *Vairiomorpha* spores were inoculated once at day 0, while BV was fed ad libitum for 12 days. Symbols on the box plot represent the maximum and minimum values (whiskers: \top \perp) and mean values (-). Different lowercase letters indicate statistical differences between treatments (one-way analysis of variance (ANOVA) with Tukey's post-hoc test, $p < 0.05$).

Vairiomorpha-infected bees, where we found a significant decrease ($p < 0.05$) in THC when compared to control and BV-fed bees at the same time points. There was a significant increase ($p < 0.05$) in THC in *Vairiomorpha*-infected bees fed BV1 or BV2 at 6- and 12-day post-infection when compared to *Vairiomorpha*-infected bees only, implying that BV promotes host defense against *Vairiomorpha* infection (Figure 3). It is worth noting that bees fed BV1 or BV2 had the highest THC levels ($p < 0.05$) compared to all other groups, but only after

12 days of exposure, indicating the effect of chronic BV exposure on THC in honey bees.

3.5. Effects on gene expression of immune-related genes

When we compared the expression levels of the four immunity-related genes investigated, defensin-1, apismín, hymenoptaecin, and PGRPS2, only apismín and hymenoptaecin were significantly downregulated ($p < 0.05$) in *Vairiomorpha*-infected

bees 6 days post-infection compared to control (Figure 4c–f), while we found a significant increase ($p < 0.05$) in the relative expression of all genes in double treatment group (*Vairimorpha* + BV1 or BV2) compared to control or *Vairimorpha*-infected or BV-fed bees (Figure 4). Furthermore, the fold change in abundance of transcripts of the four genes in the double treatment group was found to be BV concentration dependent, with higher expression in *Vairimorpha*-infected bees fed BV2 at LC₂₀ than BV1 at LC₁₀. For example, the average fold change in expression of defensin-1 and PGRPS2 in *Vairimorpha* + BV2 fed at LC₂₀ bees was 267 and 149, respectively compared to a 110- and 58-fold change in *Vairimorpha* + BV1 fed at LC₁₀ bees (Figure 4a, b). Also, there was a consistent increase in fold change of expression of all genes in bees fed BV2 at LC₂₀ versus those fed BV1 at LC₁₀. Beta-actin threshold cycles (Ct) do not significantly differ among the groups (Figure S2).

4. DISCUSSION

The current study aimed to explore the efficacy of BV against a global fungal disease, *Vairimorpha ceranae*, that has detrimental effects on bee health and causes high mortality levels. The findings support our hypothesis that BV promoted bee immunity and health while decreasing infection severity, as seen by increased bee survival rates and a significant decrease in parasite intensity. This effect was linked to an increase in THC as well as the expression of some genes that encode for AMPs.

The first step in investigating the potential role of BV against honey bee diseases was to ensure its safety to bee health. In the current study, we found that exposing newly emerged bees to BV at LC₁₀ or LC₂₀ for 12 days had no effect on honey bee survival when compared to control bees. Additionally, we found a significant increase in survival of bees with *Vairimorpha* infection and fed BV at LC₁₀ or LC₂₀, which were similar to control and BV-fed bees compared to bees with *Vairimorpha* infection, demonstrating the ability of BV to modulate the parasite virulence. Our findings are consistent

with previous research that found no adverse effect on bee survival after 6 days of oral exposure to comparable BV concentrations (100, 500, and 1,000 µg/mL) (Fernández et al. 2014). Furthermore, our findings are also consistent with a recent study that found that applying BV at the colony level at similar concentrations was not only safe for colony survival but also resulted in an increase in brood size (Seyam et al. 2022).

The antimicrobial action of BV has been shown in vitro and in vivo experiments against bacteria, viruses, and fungi (El-Seedi et al. 2020; Maitip et al. 2021; Sameh et al. 2023). In addition, the application of venom to the body surface of honey bees (Baracchi and Turillazzi 2010), as well as ants (Obin and Vander Meer 1985; Orivel et al. 2001) and wasps (Turillazzi et al. 2006) has been proposed as a means of pathogen protection. Our findings supported this hypothesis because we found a significant reduction in *V. ceranae* intensity in bees fed BV at LC₁₀ or LC₂₀, with a more profound decrease at day 12 than day 6, especially in bees fed BV at LC₂₀ compared to bees infected but not fed BV, which could explain the earlier survival results. These findings are consistent with previous research, which reported a decrease in *Nosema apis* spores and *Varroa* mites after spraying or feeding BV to infected colonies (Naglaa et al. 2020). Furthermore, BV has been reported to be an effective agent against dermatophytes caused by *Trichophyton mentagrophytes* and *Trichophyton rubrum* fungus (Yu et al. 2012). The antifungal activity of BV may be attributed to melittin, which induces reactive oxygen species resulting in the antifungal effect (Park and Lee 2010; Yu et al. 2012). Other components, such as phospholipase A2 and hyaluronidase, promote cell membrane and capillary membrane permeability and are suggested to achieve the entire antifungal action (Eiseman et al. 1982; Son et al. 2007). Raw or whole BV outperformed its primary components melittin or apamin in a dose and time-dependent manner when testing its antifungal activity (Park et al. 2018).

Hemocytes are the cellular arm of the insect's innate immune response. They are also in charge of coordinating specialized insect humoral

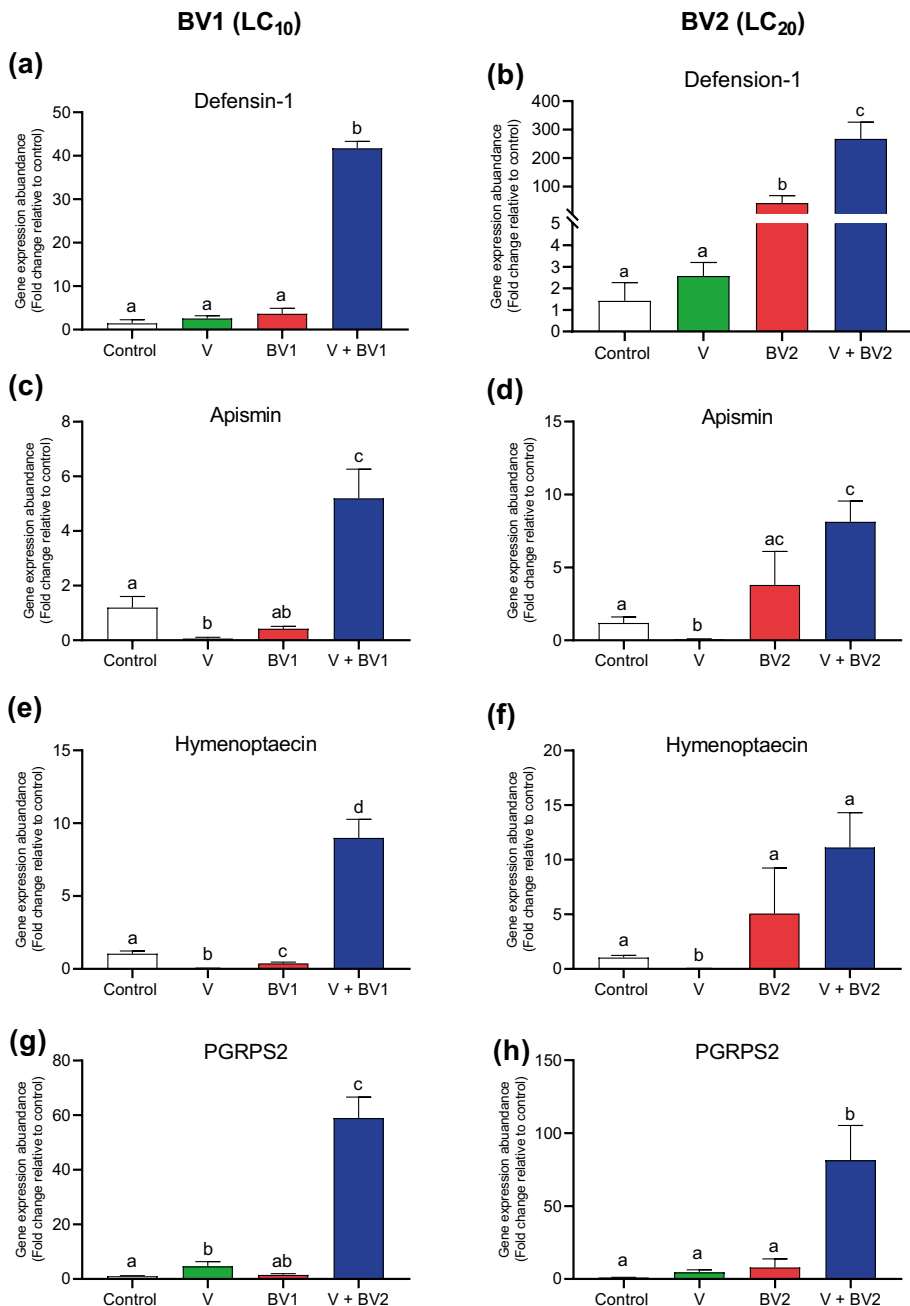


Figure 4. Fold change in gene expression abundances of some antimicrobial peptides genes (AMPs) involved in immunity (mean \pm SEM, $n=6$) in midgut tissues of adult honey bee workers in different treatments at 6 days post-infection. Bees ($n=30$ bees per cage, $n=3$ cages per treatment) were fed 10- μ l sugar syrup containing 10^5 *Vairiomorpha* (*Nosema ceranae*) spores and then fed sugar syrup containing BV1 (LC₁₀: 0.24 mg/mL) (a, c, e, g) or BV2 (LC₂₀: 0.34 mg/mL) (b, d, f, h), or a control solution. *Vairiomorpha* spores were inoculated once at day 0, while BV was fed ad libitum for 12 days. Different lowercase letters indicate statistical differences between treatments (one-way analysis of variance (ANOVA) with Tukey’s post-hoc test, $p < 0.05$).

responses during infection, such as the generation of AMPs and other effector chemicals (Strand 2008; Nehme et al. 2011; Fauvarque and Williams 2011). Previous research has reported a decrease in hemocytes count in the dampwood termite, *Zootermopsis angusticollis* in response to the entomopathogenic fungus *Metarhizium anisopliae* (Avulova and Rosengaus 2011) as well as in the stingless bee *Melipona colimana* and Africanized honey bees after *V. ceranae* infection (Macías-Macías et al. 2020; De la Mora et al. 2023). In the current investigation, we similarly found a significant drop in THC in *Vairimorpha*-infected bees; however, this trend was reversed in *Vairimorpha*-infected bees fed BV at LC₁₀ or LC₂₀ at 6- and 12-day post-infection as compared to *Vairimorpha*-infected bees alone, indicating that BV boosts bee immune system against *Vairimorpha* infection by increasing THC because hemocytes are actively involved in the production of a variety of AMPs and proteins that are released into the hemolymph in response to microbial invasion or integument damage (Hillyer 2016). These results support the substantial decrease in *Vairimorpha* intensity in *Vairimorpha*-fed BV bees that we found. It is also interesting to note that THC levels in BV-fed bees increased noticeably in comparison to control, *Vairimorpha*-infected bees that were either fed BV or both. This result emphasizes how important BV is for strengthening the bee immune system, as other studies have shown that insects exposed to pesticides and heavy metals experienced a notable decrease in THC levels (KURT and KAYIŞ 2015; Akman Gündüz 2020).

Pathogens have evolved strategies to increase their chances of survival by drastically altering the transcriptional profile and protein content of host cells (Finlay and McFadden 2006; Silmon de Monerri and Kim 2014). In the current study, we found a significant increase in the relative expression of the four AMP-encoding genes: defensin-1, apismín, hymenoptaecin, and PGRPS2 in *Vairimorpha* fed BV at LC₁₀ or LC₂₀ bees compared to control, *Vairimorpha* -infected, or BV-fed bees. Furthermore, the fold change in gene expression abundance of the four genes

was found to be BV concentration-dependent. These findings are consistent with previous research, which reported a decrease in expression of these genes in *Vairimorpha*-infected bees (Antúnez et al. 2009; Chaimanee et al. 2012) while increasing in BV-treated honey bee colonies (Seyam et al. 2022). The higher expression of AMP-encoding genes in *Vairimorpha* fed BV bees in the current study and in BV-fed bees in previous research may be explained by the bee venom enrichment in biochemicals ranging from small peptides and enzymes to biogenic amines (Hossen et al. 2016). By raising THC and AMPs, BV therefore helps triggering cellular and humoral immune responses (Dimopoulos 2003).

Recently, ten BV peptide-encoding genes (apamin, serine protease 53, Pla2-like, Pla2, secapin, venom allergen Api m 6, Melt, Mcdp, phospholipase A1 member A, transcript variant X3) were found to be differentially down-regulated in bees inoculated with black queen cell virus (BQCV) and sacbrood virus (SBV) (Al Naggat et al. 2023). As a result, one might speculate that BV treatment could modulate the expression of these BV peptide-encoding genes, reducing virus virulence. Future research will be necessary to validate this assumption and demonstrate the potential antiviral activity of BV against honey bee viral pathogens.

5. CONCLUSION

We present the first evidence of BV activity against the honey bee gut parasite *V. ceranae* under controlled laboratory conditions. BV-dietary feeding significantly reduced *Vairimorpha* intensity and increased bee survival. An increase in THC as well as the expression of AMP-encoding genes was both linked to BV activity. These findings highlight BV's potent immunomodulatory role against one of the fungal pathogens in honey bees (*V. ceranae*), and its potential role against other fungal, bacterial, and viral pathogens should be investigated.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-023-01048-2>.

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AUTHOR CONTRIBUTION

SHM: conceived and designed the experiments, carried out the experiments, and generated the data, writing materials, and methods. MK: carried out gene expression quantification. HE: reviewing and editing. YA: conceived and designed the experiments, data analysis, visualization, and writing the original draft. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval No approval of the Research Ethics Committee was required to achieve the goals of this study as the experimental work involved an unregulated invertebrate species (*Apis mellifera*).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

REFERENCES

- Akman Gündüz NE (2020) Effect of cadmium and lead on total hemocyte count of *Achroia grisella* Fabr. (Lepidoptera: Pyralidae). *Gümüşhane Üniversitesi Fen Bilim. Enstitüsü Derg.* <https://doi.org/10.17714/gumusfenbil.531102>
- Al Naggar Y, Baer B (2019) Consequences of a short time exposure to a sublethal dose of Flupyradifurone (Sivanto) pesticide early in life on survival and immunity in the honeybee (*Apis mellifera*). *Sci Rep* 9:19753. <https://doi.org/10.1038/s41598-019-56224-1>
- Al Naggar Y, Codling G, Giesy JP, Safer A (2018) Bee-keeping and the need for pollination from an agricultural perspective in Egypt. *Bee World* 95:107–112. <https://doi.org/10.1080/0005772X.2018.1484202>
- Al Naggar Y, Paxton RJ (2020) Mode of transmission determines the virulence of black queen cell virus in adult honey bees, posing a future threat to bees and apiculture. *Viruses* 12:535. <https://doi.org/10.3390/v12050535>
- Al Naggar Y, Shafiey H, Paxton RJ (2023) Transcriptomic responses underlying the high virulence of black queen cell virus and sacbrood virus following a change in their mode of transmission in honey bees (*Apis mellifera*). *Viruses* 15:1284. <https://doi.org/10.3390/v15061284>
- Al Naggar Y, Singavarapu B, Paxton RJ, Wubet T (2022) Bees under interactive stressors: the novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honey bees and increase opportunistic bacterial pathogens. *Sci Total Environ* 849:157941. <https://doi.org/10.1016/j.scitotenv.2022.157941>
- Antúnez K, Martín-Hernández R, Prieto L, Meana A, Zunino P, Higes M (2009) Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). *Environ Microbiol* 11:2284–2290. <https://doi.org/10.1111/j.1462-2920.2009.01953.x>
- Askari P, Namaei MH, Ghazvini K, Hosseini M (2021) *In vitro* and *in vivo* toxicity and antibacterial efficacy of melittin against clinical extensively drug-resistant bacteria. *BMC Pharmacol Toxicol* 22:42. <https://doi.org/10.1186/s40360-021-00503-z>
- Aufauvre J, Mismé-Aucouturier B, Viguès B, Texier C, Delbac F, Blot N (2014) Transcriptome analyses of the honeybee response to *Nosema ceranae* and insecticides. *Plos One* 9:e91686. <https://doi.org/10.1371/journal.pone.0091686>
- Avulova S, Rosengaus RB (2011) Losing the battle against fungal infection: suppression of termite immune defenses during mycosis. *J Insect Physiol* 57:966–971. <https://doi.org/10.1016/j.jinsphys.2011.04.009>
- Baracchi D, Fadda A, Turillazzi S (2012) Evidence for antiseptic behaviour towards sick adult bees in honey bee colonies. *J Insect Physiol* 58:1589–1596. <https://doi.org/10.1016/j.jinsphys.2012.09.014>
- Baracchi D, Francese S, Turillazzi S (2011) Beyond the antipredatory defence: honey bee venom function as a component of social immunity. *Toxicon* 58:550–557. <https://doi.org/10.1016/j.toxicon.2011.08.017>
- Baracchi D, Turillazzi S (2010) Differences in venom and cuticular peptides in individuals of *Apis mellifera* (Hymenoptera: Apidae) determined by MALDI-TOF MS. *J Insect Physiol* 56:366–375. <https://doi.org/10.1016/j.jinsphys.2009.11.013>
- Brosi BJ, Delaplane KS, Boots M, de Roode JC (2017) Ecological and evolutionary approaches

- to managing honeybee disease. *Nature Ecology & Evolution* 1:1250–1262. <https://doi.org/10.1038/s41559-017-0246-z>
- Chaimanee V, Chantawannakul P, Chen Y, Evans JD, Pettis JS (2012) Differential expression of immune genes of adult honey bee (*Apis mellifera*) after inoculated by *Nosema ceranae*. *J Insect Physiol* 58:1090–1095. <https://doi.org/10.1016/j.jinsphys.2012.04.016>
- de Graaf DC, Brochetto Braga MR, de Abreu RMM, Blank S, Bridts CH, De Clerck LS, Devreese B, Ebo DG, Ferris TJ, Hagendorens MM, Justo Jacomini DL, Kanchev I, Kokot ZJ, Matysiak J, Mertens C, Sabato V, Van Gasse AL, Van Vaerenbergh M (2021) Standard methods for *Apis mellifera* venom research. *J Apic Res* 60:1–31. <https://doi.org/10.1080/00218839.2020.1801073>
- De la Mora A, Morfin N, Tapia-Rivera JC, Macías-Macías JO, Tapia-González JM, Contreras-Escareño F, Petukhova T, Guzman-Novoa E (2023) The fungus *Nosema ceranae* and a sublethal dose of the neonicotinoid insecticide thiamethoxam differentially affected the health and immunity of Africanized honey bees. *Microorganisms* 11:1258. <https://doi.org/10.3390/microorganisms11051258>
- Dietemann V, Pflugfelder J, Anderson D, Charrière J-D, Chejanovsky N, Dainat B, de Miranda J, Delaplane K, Dillier F-X, Fuch S, Gallmann P, Gauthier L, Imdorf A, Koeniger N, Kralj J, Meikle W, Pettis J, Rosenkranz P, Sammataro D, Smith D, Yañez O, Neumann P (2012) *Varroa destructor*: research avenues towards sustainable control. *J Apic Res* 51:125–132. <https://doi.org/10.3896/IBRA.1.51.1.15>
- Dimopoulos G (2003) Insect immunity and its implication in mosquito-malaria interactions. *Cell Microbiol* 5:3–14. <https://doi.org/10.1046/j.1462-5822.2003.00252.x>
- Duffy C, Sorolla A, Wang E, Golden E, Woodward E, Davern K, Ho D, Johnstone E, Pflieger K, Redfern A, Iyer KS, Baer B, Blancafort P (2020) Honeybee venom and melittin suppress growth factor receptor activation in HER2-enriched and triple-negative breast cancer. *npj. Precis Oncol* 4:24. <https://doi.org/10.1038/s41698-020-00129-0>
- Eiseman JL, Von Bredow J, Alvares AP (1982) Effect of honeybee (*Apis mellifera*) venom on the course of adjuvant-induced arthritis and depression of drug metabolism in the rat. *Biochem Pharmacol* 31:1139–1146. [https://doi.org/10.1016/0006-2952\(82\)90354-9](https://doi.org/10.1016/0006-2952(82)90354-9)
- El Khoury S, Rousseau A, Lecoer A, Cheaib B, Bouslama S, Mercier PL, Demey V, Castex M, Giovenazzo P, Derome N (2018) Deleterious interaction between honeybees (*Apis mellifera*) and its microsporidian intracellular parasite *Nosema ceranae* was mitigated by administering either endogenous or allochthonous gut microbiota strains. *Front Ecol Evol* 6. <https://doi.org/10.3389/fevo.2018.00058>
- Elkobrosy DH, Aseel DG, Hafez EE, El-Saedy MA, Al-Huqail AA, Ali HM, Jebri J, Shama S, Abdelsalam NR, Elnahal ASM (2022) Quantitative detection of induced systemic resistance genes of potato roots upon ethylene treatment and cyst nematode, *Globodera rostochiensis*, infection during plant–nematode interactions. *Saudi J Biol Sci* 29:3617–3625. <https://doi.org/10.1016/j.sjbs.2022.02.045>
- El-Seedi H, Abd El-Wahed A, Yosri N, Musharraf SG, Chen L, Moustafa M, Zou X, Al-Mousawi S, Guo Z, Khatib A, Khalifa S (2020) Antimicrobial properties of *Apis mellifera*'s bee venom. *Toxins* 12:451. <https://doi.org/10.3390/toxins12070451>
- Evans JD, Aronstein K, Chen YP, Hetru C, Imler J-L, Jiang H, Kanost M, Thompson GJ, Zou Z, Hultmark D (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol Biol* 15:645–656. <https://doi.org/10.1111/j.1365-2583.2006.00682.x>
- Fauvarque M-O, Williams MJ (2011) Drosophila cellular immunity: a story of migration and adhesion. *J Cell Sci* 124:1373–1382. <https://doi.org/10.1242/jcs.064592>
- Fernández NJ, Porrini MP, Podaza EA, Damiani N, Gende LB, Eguaras MJ (2014) A scientific note on the first report of honeybee venom inhibiting *Paenibacillus* larvae growth. *Apidologie* 45:719–721. <https://doi.org/10.1007/s13592-014-0289-y>
- Finlay BB, McFadden G (2006) Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. *Cell* 124:767–782. <https://doi.org/10.1016/j.cell.2006.01.034>
- Forsgren E, Fries I (2010) Comparative virulence of *Nosema ceranae* and *Nosema Apis* in individual European honey bees. *Vet Parasitol* 170:212–217. <https://doi.org/10.1016/j.vetpar.2010.02.010>
- Fries I, Chauzat M-P, Chen Y-P, Doublet V, Genersch E, Gisder S, Higes M, McMahon DP, Martín-Hernández R, Natsopoulos M, Paxton RJ, Tanner G, Webster TC, Williams GR (2013) Standard methods for *Nosema* research. *J Apic Res* 52:1–28. <https://doi.org/10.3896/ibra.1.52.1.14>
- Gage SL, Kramer C, Calle S, Carroll M, Heien M (2018) *Nosema ceranae* parasitism impacts olfactory learning and memory and neurochemistry in honey bees (*Apis mellifera*) 1–11. <https://doi.org/10.1242/jeb.161489>
- Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhofer JH, Freitas BM, Ghazoul J, Greenleaf S, Hipolito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlof M, Seymour CL, Schuepp C, Szentgyorgyi H, Taki H, Tscharrntke T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339:1608–1611. <https://doi.org/10.1126/science.1230200>
- Higes M, García-Palencia P, Martín-Hernández R, Meana A (2007) Experimental infection of *Apis*

- mellifera* honeybees with *Nosema ceranae* (Microsporidia). *J Invertebr Pathol* 94:211–217. <https://doi.org/10.1016/j.jip.2006.11.001>
- Hillyer JF (2016) Insect immunology and hematopoiesis. *Dev Comp Immunol* 58:102–118. <https://doi.org/10.1016/j.dci.2015.12.006>
- Hossen M, Shapla U, Gan S, Khalil M (2016) Impact of bee venom enzymes on diseases and immune responses. *Molecules* 22:25. <https://doi.org/10.3390/molecules22010025>
- Hristov P, Shumkova R, Palova N, Neov B (2020) Factors associated with honey bee colony losses: a mini-review. *Vet Sci* 7:166. <https://doi.org/10.3390/vetsci7040166>
- Hu Y, Wu T, Yang E, Wu P, Lin P, Wu Y (2017) Regulation of genes related to immune signaling and detoxification in *Apis mellifera* by an inhibitor of histone deacetylation. *Nature Publishing Group* 1–14. <https://doi.org/10.1038/srep41255>
- Insolia L, Molinari R, Rogers SR, Williams GR, Chiaromonte F, Calovi M (2022) Honey bee colony loss linked to parasites, pesticides and extreme weather across the United States. *Sci Rep* 12:20787. <https://doi.org/10.1038/s41598-022-24946-4>
- Jaffé R, Veiga JC, Pope NS, Lanes ECM, Carvalho CS, Alves R, Andrade SCS, Arias MC, Bonatti V, Carvalho AT, Castro MS, Contrera FAL, Franco TM, Freitas BM, Giannini TC, Hrcncir M, Martins CF, Oliveira G, Saraiva AM, Souza BA, Imperatriz-Fonseca VL (2019) Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change. *Evol Appl* 12:1164–1177. <https://doi.org/10.1111/eva.12794>
- Jones JC (1962) Current concepts concerning insect hemocytes. *Am Zool* 2:209–246
- Khalifa SAM, Elshafiey EH, Shetaia AA, El-Wahed AAA, Algethami AF, Musharraf SG, AlAjmi MF, Zhao C, Masry SHD, Abdel-Daim MM, Halabi MF, Kai G, Al Naggar Y, Bishr M, Diab MAM, El-Seedi HR (2021) Overview of bee pollination and its economic value for crop production. *InSects* 12:688. <https://doi.org/10.3390/insects12080688>
- Kuhn-Nentwig L (2003) Antimicrobial and cytolytic peptides of venomous arthropods. *Cellular and Molecular Life Sciences (CMLS)* 60:2651–2668. <https://doi.org/10.1007/s00018-003-3106-8>
- Kurt D, Kayış T (2015) Effects of the pyrethroid insecticide deltamethrin on the hemocytes of *Galleria mellonella*. *Turkish J Zool* 39:452–457. <https://doi.org/10.3906/zoo-1405-66>
- Lima MAP, Cutler GC, Mazzeo G, Hrcncir M (2022) Editorial: the decline of wild bees: causes and consequences. *Front Ecol Evol* 10. <https://doi.org/10.3389/fevo.2022.1027169>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
- Macías-Macías JO, Tapia-Rivera JC, De la Mora A, Tapia-González JM, Contreras-Escareño F, Petukhova T, Morfin N, Guzman-Novoa E (2020) *Nosema ceranae* causes cellular immunosuppression and interacts with thiamethoxam to increase mortality in the stingless bee *Melipona colimana*. *Sci Rep* 10:17021. <https://doi.org/10.1038/s41598-020-74209-3>
- Maitip J, Mookhploy W, Khorndork S, Chantawannakul P (2021) Comparative study of antimicrobial properties of bee venom extracts and melittins of honey bees. *Antibiotics* 10:1503. <https://doi.org/10.3390/antibiotics10121503>
- Mura A, Puseddu M, Theodorou P, Angioni A, Floris I, Paxton RJ, Satta A (2020) Propolis consumption reduces *Nosema ceranae* infection of European honey bees (*Apis mellifera*). *InSects* 11:124. <https://doi.org/10.3390/insects11020124>
- Naglaa FB, Wael MM, Mohamed EH (2020) Controlling of some honeybee *Apis mellifera* (Hymenoptera: Apidae) colonies diseases by bee venom. *Egypt J Plant Prot Res Inst* 4:1139–1150
- Nehme NT, Quintin J, Cho JH, Lee J, Lafarge M-C, Kocks C, Ferrandon D (2011) Relative roles of the cellular and humoral responses in the *Drosophila* host defense against three gram-positive bacterial infections. *PLoS ONE* 6:e14743. <https://doi.org/10.1371/journal.pone.0014743>
- Obin MS, Vander Meer RK (1985) Gaster flagging by fire ants (*Solenopsis* spp.): functional significance of venom dispersal behavior. *J Chem Ecol* 11:1757–1768. <https://doi.org/10.1007/BF0102125>
- Orivel J, Redeker V, Le Caer J-P, Krier F, Revol-Junelles A-M, Longeau A, Chaffotte A, Dejean A, Rossier J (2001) Poneracins, New antibacterial and insecticidal peptides from the venom of the ant *Pachycondyla goeldii*. *J Biol Chem* 276:17823–17829. <https://doi.org/10.1074/jbc.M100216200>
- Park C, Lee DG (2010) Melittin induces apoptotic features in *Candida albicans*. *Biochem Biophys Res Commun* 394:170–172. <https://doi.org/10.1016/j.bbrc.2010.02.138>
- Park J, Kwon O, An H-J, Park KK (2018) Antifungal effects of bee venom components on *trichophyton rubrum*: a novel approach of bee venom study for possible emerging antifungal agent. *Ann Dermatol* 30:202. <https://doi.org/10.5021/ad.2018.30.2.202>
- Phiri BJ, Fèvre D, Hidano A (2022) Uptrend in global managed honey bee colonies and production based on a six-decade viewpoint, 1961–2017. *Sci Rep* 12:21298. <https://doi.org/10.1038/s41598-022-25290-3>
- Rady I, Siddiqui IA, Rady M, Mukhtar H (2017) Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. *Cancer Lett* 402:16–31. <https://doi.org/10.1016/j.canlet.2017.05.010>
- Sameh A, Gouda AA, Elmligy E, Hatem H, Sadek SS, Ahmed O, El Amir A (2023) Bee venom as an alternative for antibiotics against *Staphylococcus aureus* infections. *Sci Rep* 13:6436. <https://doi.org/10.1038/s41598-023-33536-x>

- Seyam H, Metwally AAA, El-Deeb AH, El-Mohandes S, Badr MS, Abd-El-Samie EM (2022) Effect of honeybee venom and Egyptian propolis on the honeybee (*Apis mellifera* L.) health *in vivo*. *Egypt J Biol Pest Control* 32:78. <https://doi.org/10.1186/s41938-022-00580-0>
- Silmon de Monerri NC, Kim K (2014) Pathogens hijack the epigenome. *Am J Pathol* 184:897–911. <https://doi.org/10.1016/j.ajpath.2013.12.022>
- Simone-Finstrom M, Spivak M (2010) Propolis and bee health: the natural history and significance of resin use by honey bees. *Apidologie* 41:295–311. <https://doi.org/10.1051/apido/2010016>
- Son D, Lee J, Lee Y, Song H, Lee C, Hong J (2007) Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacol Ther* 115:246–270. <https://doi.org/10.1016/j.pharmthera.2007.04.004>
- Stow A, Briscoe D, Gillings M, Holley M, Smith S, Leys R, Silberbauer T, Turnbull C, Beattie A (2007) Antimicrobial defences increase with sociality in bees. *Biol Lett* 3:422–424. <https://doi.org/10.1098/rsbl.2007.0178>
- Strand MR (2008) The insect cellular immune response. *Insect Sci* 15:1–14. <https://doi.org/10.1111/j.1744-7917.2008.00183.x>
- Tauber OE, Yeager JF (1935) On total hemolymph (blood) cell counts of insects I. Orthoptera, Odonata, Hemiptera, and Homoptera. *Ann Entomol Soc Am* 28:229–240. <https://doi.org/10.1093/aesa/28.2.229>
- Turillazzi S, Mastrobuoni G, Dani FR, Moneti G, Pieraccini G, la Marca G, Bartolucci G, Perito B, Lambardi D, Cavallini V, Dapporto L (2006) Dominulin A and B: two new antibacterial peptides identified on the cuticle and in the venom of the social paper wasp *Polistes dominulus* using MALDI-TOF, MALDI-TOF/TOF, and ESI-ion trap. *J Am Soc Mass Spectrom* 17:376–383. <https://doi.org/10.1016/j.jasms.2005.11.017>
- Vinson SB (1993) Suppression of the insect immune system by parasitic hymenoptera, in: insect immunity. Springer Netherlands, Dordrecht, pp. 171–187. https://doi.org/10.1007/978-94-011-1618-3_12
- Wehbe R, Frangieh J, Rima M, El Obeid D, Sabatier J-M, Fajloun Z (2019) Bee venom: overview of main compounds and bioactivities for therapeutic interests. *Molecules* 24:2997. <https://doi.org/10.3390/molecules24162997>
- Weis WA, Ripari N, Conte FL, da Honorio MS, Sartori AA, Matusci RH, Sforzin JM (2022) An overview about apitherapy and its clinical applications. *Phytomed Plus* 2:100239. <https://doi.org/10.1016/j.phyplu.2022.100239>
- Wilson-Rich N, Spivak M, Fefferman NH, Starks PT (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu Rev Entomol* 54:405–423. <https://doi.org/10.1146/annurev.ento.53.103106.093301>
- Yang X, Cox-Foster DL (2005) Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. *Proc Natl Acad Sci* 102:7470–7475. <https://doi.org/10.1073/pnas.0501860102>
- Yu A-R, Kim J-J, Park G-S, Oh S-M, Han C-S, Lee M-Y (2012) The antifungal activity of bee venom against dermatophytes. *J Appl Biol Chem* 55:7–11. <https://doi.org/10.3839/jabc.2011.052>
- Zaslouff M (2002) Antimicrobial peptides of multicellular organisms. *Nature* 415:389–395. <https://doi.org/10.1038/415389a>

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